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Reduced preabsorptive insulin response in aged rats: differential effects of amphetamine and arginine-vasopressin

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Abstract

The experiments presented here have been designed to investigate whether the age-related attenuation of the vagal reactivity to emotional stressors and its modulation by amphetamine (Amph) or arginine-vasopressin (AVP) can be generalized for other physiological response patterns. We therefore studied the vagal control of the endocrine pancreas during food intake. Young (3 months old) and aged (27 months old) male Wistar rats were provided with permanent cardiac catheters allowing free movement and repeated, stress-free blood sampling. The vagally mediated preabsorptive insulin response (PIR) in relation to food intake as seen in young rats was reduced in aged ones. Blood glucose increments were the same at both ages. Administration of Amph (0.5 mg/kg; s.c.) 30 min before, or AVP (10 μg/kg; s.c.) 60 min before presentation of a test meal led to an elevation of the magnitude of insulin secretion in young rats but reduced the response in aged rats. Moreover, the PIR was not reinstated in aged rats. Blood glucose increments were not influenced by the treatments. The results are interpreted in terms of age-related general reduction of parasympathetic reactivity. The differential effect of amphetamine and AVP treatment on the insulin response suggests that the central aminergic or peptidergic drive of vagal output to the endocrine pancreas is also age-related.

Introduction

Physiological and neuroendocrine stress responses are markedly affected by age in animals and man [5,18,19,21,22]. Previous studies in our laboratory have shown an age-related reduction in the initial bradycardia to a conditioned emotional stress (fear of inescapable footshock) [19]. Since this response could be blocked completely by atropine (Buwalda, unpublished results) it is probably vagally mediated and a diminution of stress-related parasympathetic control of cardiac functioning during aging was suggested [19]. Administration of the psychostimulant amphetamine (Amph) appeared to reinstate the bradycardia response in aged rats [18]. In a similar experimental design the effect of arginine-8-vasopressin (AVP) was investigated. AVP appeared to intensify the bradycardia response in young adult rats [3] and this neuropeptide restored the bradycardia in 14 month-old rats also [18].

The question was raised as to whether the age-related diminution of vagal reactivity and its aminergic or peptidergic modulation can be generalized for other physiological response patterns.

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During feeding, plasma insulin level increases in the first minute of food presentation before an elevation in peripheral glucose can be observed [2,24]. It has been repeatedly demonstrated that this preabsorptive insulin response (PIR) is of cephalic origin [2,8,20,25], and can be abolished by vagotomy [11,12,26] or atropine methyl-nitrate [2,25]. All of these results were obtained in Wistar rats, and, although we do not show again that vagal activation provokes a PIR, it is reasonable to assume that the insulin response in these animals is mediated by the vagus nerve.

The present experiments were designed to investigate the pattern of the cephalic insulin secretion in young and aged Wistar rats during the presentation of the non-aversive physiological stimulus of food. In addition, the modulating effects of Amph and AVP on this response were studied.

Materials and Methods

Animals

Young (3 months old) and aged (28 months old) male Wistar rats were kept individually in perspex cages (25 × 25 × 30 cm) at a room temperature of 20 ± 2°C and had continuous access to standard carbohydrate-rich food (Hope Farms Laboratory chow) and water unless otherwise stated. At the beginning of the experiments the young rats ($n = 8$) weighed 289 ± 2 g and the aged rats ($n = 6$) 417 ± 9 g. They were housed in a 12-h light-dark cycle (the lights were on from 00.00 to 12.00 h).

Experimental procedure

The rats were trained to consume a test meal consisting of 2 g of ground rat chow mixed with 2 ml water offered in a porcelain dish. Before the onset of the experiments the animals were deprived of food for 4 h between 09.00 and 13.00 h, i.e. in the period of high spontaneous food intake. The animals started to eat immediately after presentation of food. Only those rats that consumed the test meal within 4 min participated in the experiments. The tests were performed between 13.00 and 16.00 h on separate days with an interval of at least 3 days. Young and aged rats were tested in randomized order to prevent differences in fasting period. The first experiment consisted of the presentation of a test meal to determine whether the PIR was reduced in the aged animals when compared to the young ones. In the second experiment the effect of treatment with *d*-amphetamine sulphate (Amph, OPG, Utrecht, The Netherlands) or AVP on plasma insulin and blood glucose levels after a test meal was compared with that of a saline treatment. Animals were subcutaneously (s.c.) injected with saline 30 min before meal presentation. Three days later Amph was injected s.c. in a dose of 0.5 mg/kg b.w. dissolved in saline (0.5 mg Amph/ml) 30 min before the test meal. In a further test, AVP was administered s.c. in a dose of 10 μg/kg dissolved in saline (10 μg AVP/ml) 60 min before the presentation of the meal. The selection of these doses of Amph and AVP was based on the results of the previous studies of young and aged rats' vagally mediated cardiac response to emotional stress [18].

Blood sampling

The rats were provided with a permanent catheter in the right atrium inserted via the right jugular vein and externalized on the head of the rat. This catheter allows repeated blood sampling in the unrestrained and undisturbed rat [23]. Surgery was performed under complete ether anesthesia. Before the start of the experiments the animals were handled for at least a week to accustom them to the sampling procedure. Blood samples of 0.25 ml were taken in vials containing 5 μl heparin (500 IU). In order to obtain relatively stable basal blood insulin and glucose levels, the meal tests were performed after a fasting period of 4 h. Blood samples were taken at 0 (start of meal ingestion), 1, 2, 3 and 5 min. Withdrawn blood was replaced by transfusion of heparinized blood (25 IU per ml) of a donor rat fed ad libitum in order to minimize the changes in blood volume. A transfusion of 0.5 ml blood was given at −5 and between 3 and 5 min. After the 5th-min sample a last transfusion of 0.25 ml was given.

Insulin and glucose determinations

Blood samples were immediately chilled and blood glucose was measured from whole blood by a ferrycyanide method with a Technicon analyzer. The remaining blood was centrifuged at 4°C. The plasma was stored at −20°C until analysis. Plasma insulin was measured by radioimmunoassay (NOVO-Denmark) using rat insulin as a standard, ^{125}I-labelled porcine insulin and antiporcine insulin guinea pig serum M 8309. Samples (25 μl) were measured in duplicate. Bound and free ^{125}I-labelled insulin were separated by polyethylene glycol (23.75% w/w in water) precipitation. The coefficient of variation of the immunoassay was $< 8\%$.

Statistical analysis and calculation

The data were expressed as mean delta increases to basal levels at $t = 0$ min ± standard errors of the mean (SEM). Statistical analysis was performed by using a Multivariate Analysis of Variance (MANOVA) followed by post-hoc t-tests. The paired t-test was used for comparisons within individuals. The criterion of significance was set at $P \leqslant 0.05$.

Results

Table I shows the mean basal insulin and glucose levels in young and aged rats. No difference was found between untreated rats. There was also no significant change of basal levels following administrations of saline, Amph or AVP.

TABLE I

Basal levels of plasma insulin and blood glucose in young and aged rats

Treatment	Insulin μU/ml plasma		Glucose mg/dl blood	
	young	aged	young	aged
None	54±6 *	69±5.4	95±1.9	88±2.8
Saline	67±4.7	63±2.7	96±2.1	95±2.5
Amph	66±4.2	64±4.8	93±2.1	97±4.1
AVP	69±4.7	68±7.4	98±3.1	98±3.1

* Mean ± SEM.

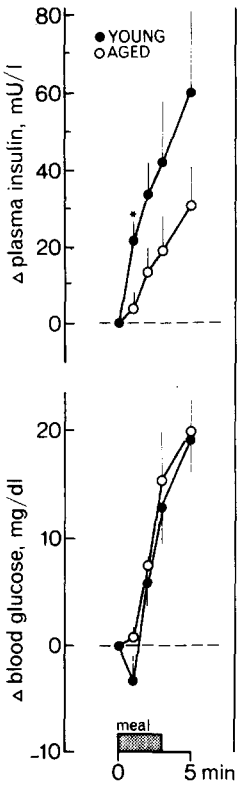

Fig. 1. Plasma insulin and blood glucose responses to a test meal in young (3 months old) ●———● and aged (27 months old) ○———○ male Wistar rats. Means ± SE are from 6–8 rats per group. * $P < 0.05$ young vs. aged group (two-tailed t-test). The time 0 min represents the onset of the meal consumption (shaded area).

Fig. 1 shows the changes in plasma insulin and blood glucose levels in response to a test meal in young and aged rats. A significant rise in plasma insulin was observed in the first minute after the start of the meal in the young rats ($P < 0.05$) with a progressive rise in the subsequent minutes. In the aged rats the early insulin increment was absent. During the following minutes a slower and smaller rise occurred in the plasma insulin levels when compared to young rats. The meal-induced rise in plasma insulin reached significance only in the 5th min. Blood glucose levels rose sharply after the 2nd min of the test meal in both young and aged rats.

Fig. 2 shows plasma insulin and blood glucose changes to the test meal after treatment with

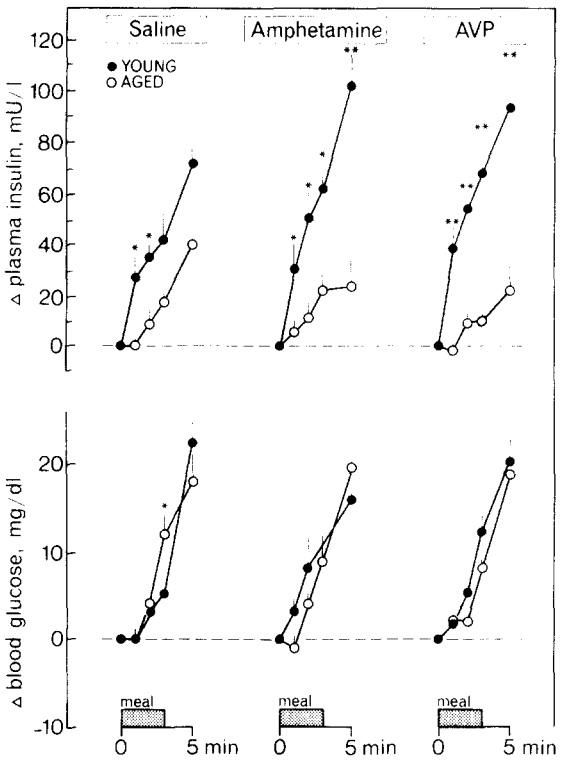

Fig. 2. Plasma insulin and blood glucose responses to a test meal in young (3 months old) ●———● and aged (27 months old) ○———○ male Wistar rats after subcutaneous treatment with saline, amphetamine (0.5 mg/kg) or AVP (10 μg/kg) 30, respectively 60 min before onset of the meal. Means ± SE are from 5–6 rats per group. * $P < 0.05$ ** $P < 0.01$ young vs. aged group (two-tailed t-test). The time 0 min represents the onset of the meal consumption (shaded area).

saline, Amph or AVP. Under all conditions a significant rise in insulin levels was observed in the 1st min in the young rats ($P < 0.05$). Differences between young and aged rats in the shape of the response curves could be seen after the 2nd min of the meal. In young animals higher insulin values were found following amphetamine and AVP treatment. In contrast, insulin release to the test meal in aged rats was reduced and showed a significant increment only on the 3rd min. Administration of the drug or peptide enhanced, albeit not significantly, the magnitude of the insulin response in young animals but slightly suppressed the response in aged rats. This differential effect of Amph and AVP on insulin responses in both groups is indicated by the MANOVA with repeated measurements for the time points after start of the meal. There was no interaction between age and sampling time after saline treatment ($F(3,36) = 0.22$, $P = 0.88$), indicating that besides the difference in insulin secretion in the 1st min, insulin levels increased in a similar way in young and aged rats. This interaction, however, became highly significant after treating young and aged rats with Amph ($F(3,24) = 5.86$, $P = 0.004$). Pretreatment with AVP had a similar effect ($F(3,27) = 2.71$, $P = 0.06$). Post-hoc t-test revealed that these interactions were caused by a difference in the magnitude of the insulin response between young and aged rats following treatment with Amph or AVP.

Pre-meal differences in blood glucose levels never reached significance before the 2nd min after presentation of the meal. As regards the glucose responses, MANOVA did not indicate a difference between the young and old animals after each of the treatments.

Discussion

The first major finding of these experiments is a reduced preabsorptive insulin response in aged in comparison to young rats. One of the possible explanations is a diminished activation of vagal output during ingestion of a meal in aged rats. Alternatively, the pancreatic B cell response to vagal activation may be reduced due to changes in cholinergic or other receptors. The reduced PIR in aged rats did not result in different glucose increments compared with the young during the first 5 min after start of the meal. The absence of alterations in blood glucose levels in the aged rats with diminished insulin response is surprising. As well as the impaired insulin response, one would also expect changes in glucose response on the basis of decreased glucose tolerance during aging [6,9]. However, studies with subdiaphragmatic vagotomized rats suggest that the early phase of the glucose response is not affected by this vagal section [26]. The possibility

remains open whether the impairment is revealed after a longer delay, i.e. beyond the measurements performed in the present investigation.

Age-related decreases in parasympathetically mediated cardiovascular responses were found in the magnitude of the baroreceptor reflex-induced bradycardia [21], the diminution of bradycardia resulting from an emotional stress in rats [19] and reduced heart rate variability in man [5]. The present results may be viewed as an extension of this: aging resulting in a reduction of a vagally mediated response in the rat to an unconditioned, non-aversive physiological stimulus such as eating a small meal.

The results of the second experiment indicate that there is a differential effect of Amph and AVP on insulin secretion in young and aged rats. In the young animals both substances mainly affected the magnitude of insulin secretion. In aged rats the peak insulin secretion is blunted and neither Amph nor AVP treatment reinstated the PIR.

The major effect of Amph is to facilitate the release of biogenic amines through presynaptic mechanisms [15]. A direct peripheral effect of the drug on insulin secretion would then be expected to be of an inhibitory nature [1,4]. It is therefore probable that the potentiation of the PIR in young rats is caused by enhancement of vagal drive by an action within the central nervous system. Since hypothalamic monoamines are closely associated with the occurrence of the PIR [8] Amph, by acting through these central sites, might modulate the vagal output from the brain stem. The absence of an effect of Amph on insulin secretion in aged rats may be due to a shift in the balance between inhibitory sympathetic and stimulatory parasympathetic neural input to the pancreas during the process of aging. The inhibitory action of adrenergic neurotransmitters on insulin secretion may be increased in the aged rat. The preabsorptive insulin response was also potentiated by the neuropeptide AVP in young, but not in aged rats. Although AVP may directly affect hepatic glucose release [27], the absence of differences in the blood glucose levels before food presentation suggests that other mechanisms are involved. There is disagreement about the direct effect of AVP on B cells. Malaisse et al. [14] found no effect of AVP on insulin secretion by B cells in vitro. More recently Gao et al. [7] showed that AVP produced a dose-dependent amplification of glucose-induced insulin release in normal mouse islets. Since AVP had little effect on cAMP levels, but increased inositol phosphate levels in islet cells, it was concluded that this AVP-evoked amplification involves a stimulation of phosphoinositide metabolism. The differential effect of AVP on insulin secretion in young and aged rats may therefore be based upon a difference in the sensitivity of the B cell second messenger system. AVP also acts on central nervous systems [28], therefore an age-related change in central vasopressinergic systems might also be responsible for the differential effect of AVP on the PIR.

While the drug and the peptide did not enhance the insulin response in aged animals, both treatments did restore the bradycardiac emotional stress response in aged rats in doses as used in this experiment [18]. Several factors may contribute to this difference. An increased behavioural arousal by Amph and AVP [10,15,28] probably plays a permissive role in the stress-evoked bradycardia [18]. So far, there is no evidence that behavioural arousal is a causal factor in eliciting the PIR. An alternative explanation may be based upon a differential innervation pattern of the pancreatic B cells and the heart by the vagus nerve. Innervation of the former originates mainly in the dorsal motor nucleus of the vagus [13]. Vagus cardiac preganglionic cells have been localized in the nucleus ambiguus, the dorsal motor nucleus of the vagus and in an intermediate zone between these two nuclei [16,17]. One can also not exclude the possibility that the responsiveness of the B cell cholinergic (muscarinic) receptors to vagal stimulation is exclusively diminished.

In summary, the present study suggests a decrease in parasympathetic influence on the early insulin response to a meal in aged rats. In contrast to a condition with an aversive stimulus, Amph and AVP were not able to reinstate the vagal responses in aged rats during the nonaversive stimulus presented here.

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